UNIVERSITY OF COPENHAGEN

Development of a PCR method for verification of Salmonella Dublin

Hansen, Flemming; Jacobsen, T.; Persson, S.; Olsen, John Elmerdahl; Mansdal, S.

Published in:
22nd International ICFMH Symposium Food Micro 2010

Publication date:
2010

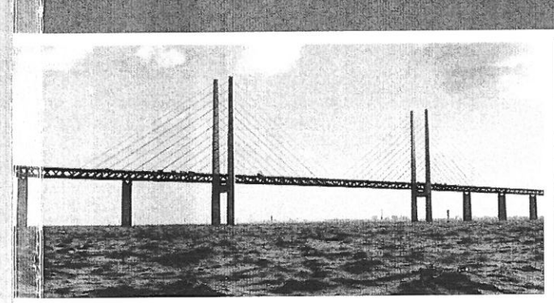
Document version
Publisher's PDF, also known as Version of record

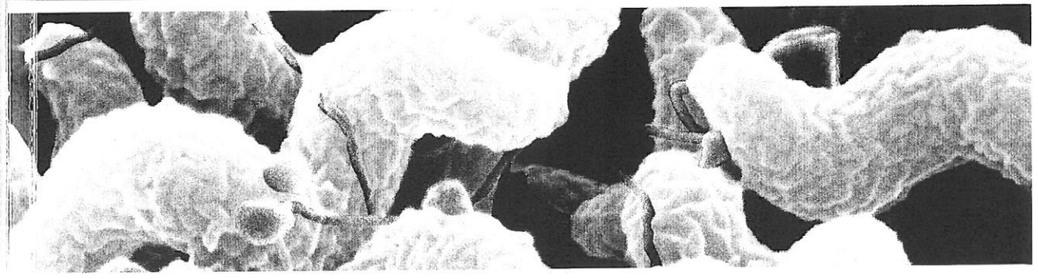
Citation for published version (APA):
Hansen, F., Jacobsen, T., Persson, S., Olsen, J. E., & Mansdal, S. (2010). Development of a PCR method for verification of *Salmonella* Dublin. In *22nd International ICFMH Symposium Food Micro 2010* (pp. 185). [PEC1.67]

Download date: 05. May. 2023

22nd International ICFMH Symposium

Food Micro 2010





Final Programme & Abstract Book

:to bacillus fermentum in

omyces cerevisae and Lac-
growth temperature (T) on
tions system (ODEs), which
wth, stationary, and death
thetical four-step reaction
through a *quorum* sensing
rially clarified and adjusted
ast (10^6CFU/mL) and lacto-
nsion in Neubauer chamber
entific, Model G-27, U.S.A.)
asts and lactobacilli counts
mphenicol (100mg/L, each)
5. Growth parameters were
ial equation solver ODE15s,
i chemical model described
ıential to stationary phase,
ctobacilli; while k_i the rate
ıtion. A secondary growth
e significant ($p<0.1$). These

/phimurium DT104 in fresh
:ection of these organisms
method, being rapid, user-
ce was undertaken, includ-
samples. The regular assay
104.
nd 8 strains of G÷ bacteria
ns were cultured overnight
DNA extraction, PCR and
Skin from chicken, slices of
) cfu per 25 g) using three
ıy 0.1 ml was inoculated in
the rim of the growth zone

la. All 59 *Salmonella* were
ting samples directly from
ı number of spiked samples
ıple material directly taken
ates.

PEC1.67 **Development of a PCR method for verification of *Salmonella* Dublin**

Flemming Hansen (1), T Jacobsen (1), S Persson (2), JE Olsen (3), S Mansdal (1)
(1) Danish Meat Research Institute, Roskilde, Denmark
(2) Statens Serum Institut, Copenhagen S, Denmark
(3) University of Copenhagen, Faculty of Life Sciences, Frederiksberg C, Denmark

In Denmark, special rules regarding absence of *Salmonella* Dublin in beef marketed as fresh meat is agreed on by the Danish food authorities and the meat sector. Thus, the Danish meat sector needs a method for rapid identification of *S.* Dublin in order to avoid distributing contaminated meat. Consequently, a new method for verification of *S.* Dublin based on PCR was developed.

Briefly, 9 different primer pairs targeting the virulence plasmid or the *rfbE* and *fliC* genes were designed for specific identification of *S.* Dublin. The primers were evaluated using a real time SYBR-Green PCR against a test panel of 37 different bacterial strains including 13 strains of *S.* Dublin. Two primer pairs showed 100% specificity and for these, probes were designed in order to develop a robust real-time PCR analysis. The two primer/probe systems were optimised using different commercially available PCR master mixes.

The two optimised PCR protocols were further evaluated using a broader test panel of 92 bacterial strains including 46 *S.* Dublin strains, strains of other *Salmonella* known to harbour the virulence plasmid and other *Salmonella* belonging to the D serogroup (O:9).

Both primer/probes systems were 100% specific for *S.* Dublin, but as one system had a slightly better Limit of Detection and gave the highest fluorescence signals, this system was selected for the final protocol.

In order to obtain approval from NordVal, the final protocol was validated according to the NordVal protocol. Briefly, a comparative study comprising 50 strains of *S.* Dublin, 20 strains of other *Salmonella* and 10 *Enterobacteriaceae* was carried out in comparison to the slide agglutination according to the Kauffmann-White Scheme. The comparative study showed 100% agreement between the *S.* Dublin PCR and the reference method. Subsequently, a collaborative trial will be undertaken in march 2010. Approximately 10 laboratories will receive each 20 samples of unknown *Salmonella* serotypes for verification using the new *S.* Dublin PCR analysis. The results will be compared with the results obtained by slide agglutination at the Danish Reference Laboratory.

PEC1.68 **RNA extraction and separation of pathogenic bacteria from food**

Jette Kjeldgaard (1), S Henriksen (2), MT Cohn (1), S Aabo (2), H Ingmer (1)
(1) Univeristy of Copenhagen, Department of Veterinary Disease Biology, Frederiksberg, Denmark
(2) The National Food Institute, DTU, Mørkhøj, Denmark

Numerous studies focus on the expression of stress or virulence genes of pathogenic bacteria under environmental stress in laboratory media and growth or survival of pathogens has often been evaluated in all kinds of food products. Despite this, few studies focus on expression of genes in pathogens present in food products. This study seeks to develop methods to stabilize and extract RNA suitable for quantitative gene expression studies of pathogens directly from meat products. The developed methods include a conventional column based method, supported by sample preparation by immobilization of bacteria with metal hydroxides and separation by centrifugation. The optimized column based method proved to be efficient in extracting bacterial RNA of high integrity and with minimal inhibition of the subsequent Reverse-Transcription Real-Time PCR (qRT-PCR) reaction directly from minced meat.

Sample preparation with metal hydroxides has been used for extracting bacteria from various matrices, including minced meat, with binding percentages of 50-99 % for different bacteria, including food spoilage bacteria and pathogens. This method simplified the removal of a large part of food particles and food-associated inhibitors before the RNA was extracted. We found that we can obtain bacterial RNA of high quality and integrity from a complex food matrix, and it is thereby possible to measure gene expression of bacteria directly from minced meat with qRT-PCR. This knowledge is very important for our understanding of how bacterial pathogens survive in food products and subsequently infect the consumer.